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Bacteriophage-Induced Functions in *Escherichia coli* K(λ) Infected with rII Mutants of Bacteriophage T4

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ABSTRACT

RUTBERG, BLANKA (Karolinska Institutet, Stockholm, Sweden), AND LARS RUTBERG. Bacteriophage-induced functions in *Escherichia coli* K(λ) infected with rII mutants of bacteriophage T4. J. Bacteriol. 91:76–80. 1966.—When *Escherichia coli* K(λ) was infected with rII mutants of phage T4, deoxycytidine triphosphatase, one of the phage-induced early enzymes, was produced at initially the same rate as in r^+-infected cells. Deoxyribonuclease activity was one-third to one-half of that of r^+-infected cells. This lower deoxyribonuclease activity was observed also in other hosts or when infection was made with rI or rIII mutants. Presence of chloramphenicol did not allow a continued synthesis of phage deoxyribonucleic acid in rII-infected K(λ). No phage lysozyme was detected nor was any antiphage serum-blocking antigen found in rII-infected K(λ). It is suggested that the rII gene is of significance for the expression of phage-induced late functions in the host K(λ).

The structure of the rII gene of phage T4 is known in its most intimate details (4), but the physiology of the rII gene is still largely unknown (9, 11, 13, 20). The aim of the present work was to study some phage-specific products induced by infection with T4rII of its nonpermissive host *Escherichia coli* K(λ). Production of deoxycytidine triphosphatase and deoxyribonuclease was chosen to represent early functions of the phage genome; among late functions, phage lysozyme and antiphage serum-blocking antigen were studied. In the experiments concerning deoxyribonuclease activity, rI and rIII mutants were included.

MATERIALS AND METHODS

Bacterial strains. *E. coli* B, *E. coli* Bc (*E. coli* B cured from its defective prophage) *E. coli* K (C 600 of Appleyard (3)), and *E. coli* K(λ). The bacterial strains were kindly provided by G. Bertani.

Phage strains. T4D, T4D r47 (rII, cistron A), T4D r73 (rII, cistron B), and T4D r48 (rI) (8) were used. In addition, an rIII mutant was isolated in our laboratory from a stock of T4D grown on *E. coli* K with 50 μg of 5-bromouracil added per ml. The mutant was defined by giving r plaques on B and wild-type (w) plaques on K(λ). The plaque morphology of the r mutants on the host bacteria employed is shown in Table 1.

Bacteria and phage were assayed by the top-layer agar method (1) on plates containing tryptone (Difco), 10 g; NaCl, 5 g; and agar (Difco), 12 g; per liter of distilled water. The top-layer agar contained 7 g of agar per liter.

Extracts from infected bacteria were prepared in the following way. An overnight culture of *E. coli* was diluted to 5×10^7 bacteria per milliliter in the Casamino Acids medium of Fraser and Jerrel (10) with glucose (10 g per liter) instead of glycerol. For K strains, 5 μg of vitamin B_1 per ml was added. The bacteria were grown with aeration at 37 C until they reached a concentration of 5×10^8 cells per milliliter; 10 μg/ml of L-tryptophan was added. The bacteria were infected at an effective multiplicity of 5 to 7. At various times, 12-ml samples were removed and poured onto 6 g of frozen medium. The cells were collected by centrifugation at 4 C and were suspended in 2 ml of distilled water. The pellets were kept at −20 C until used (1 to 3 days). The bacteria were disintegrated in an X-press (7). Before incubation, the extracts were gently thawed while stirring and were centrifuged for 15 min at 10,000 × *g*. The centrifugation did not change the activity of any of the enzymes measured. The supernatant fluid was used for the enzyme assays and for determinations of the serum-blocking antigen power.

The protein content of the extracts was determined by the ninhydrin method of Stegemann (17). In each series of extracts from an infected culture, the samples contained, within ±10%, the same amount of protein (0.6 to 1.6 mg/ml).

Deoxycytidine triphosphatase activity was determined according to Wiberg et al. (21). Deoxycytidine triphosphate was purchased from P-L Biochemicals (Pabst), Milwaukee, Wis.

Deoxyribonuclease activity was measured as described by Stone and Burton (19): 0.3 ml of extract, 14 mM phosphate buffer (*p*H 7.4), 36 mM $MgSO_4$, and 1 mg of calf thymus deoxyribonucleic acid (DNA) were incubated in a total volume of 1.4 ml for 2 hr.

TABLE 1. *Plaque morphology of r mutants on host bacteria*

r mutant	*Escherichia coli* strain			
	B	Bc	K(λ)	K
rI	r	r	r	r
rII	r	w	—	w
rIII	r	w	w	w

The reaction was stopped by adding 0.1 volume of 2.5 N perchloric acid. After 30 min of incubation at 4 C, the mixtures were centrifuged and deoxyribose was determined in the supernatant fluids according to Burton's modification of Dische's method (5). DNA was obtained from Worthington Biochemical Corp., Freehold, N.J.

Phage lysozyme was assayed with chloroform-treated *E. coli* B as substrate, as described by Sekiguchi and Cohen (16). The readings were made in a Unicam colorimeter Sp. 1300 with filter no. 5. One lysozyme (endolysin) unit is defined as the amount giving a decrease in turbidity of one scale unit per minute.

Antiphage serum-blocking antigen was measured by the method of DeMars (6).

Incorporation of thymidine-2-C^{14} was assayed as acid-insoluble material on Millipore filters (18). The countings were made in a windowless flow counter. Thymidine-2-C^{14} was purchased from New England Nuclear Corp., Boston, Mass.

RESULTS

In Fig. 1 is presented one experiment where deoxycytidine triphosphatase activity was measured in K(λ) infected with T4r$^+$ and T4rII. The same initial rate of deoxycytidine triphosphatase production was found in both samples. Maximal activity was found at 12 min after infection with T4rII, whereas the activity of T4r$^+$-infected cells increased until 16 min. The activity found in r$^+$-infected cells agrees well with that reported earlier for T2-infected *E. coli* B (12).

Deoxyribonuclease activity was measured in *E. coli* K(λ), K, B, and Bc infected with T4r$^+$ or various r mutants thereof (Table 2).

As a rule, r-infected cells showed a smaller increase in deoxyribonuclease activity than did r$^+$-infected ones. This is thus related to the r character as such, and possibly also to the properties of the host (e.g., rIII in Bc). A large number of experiments have been performed to confirm these findings. It has been reported previously that r-infected *E. coli* B shows less deoxyribonuclease activity than r$^+$-infected *E. coli* B (14).

Supernatant fluids from r-infected bacteria showed less deoxyribonuclease activity than did supernatant fluids from r$^+$-infected bacteria.

Ribonuclease treatment increased deoxyribonuclease activity to the same extent in all samples.

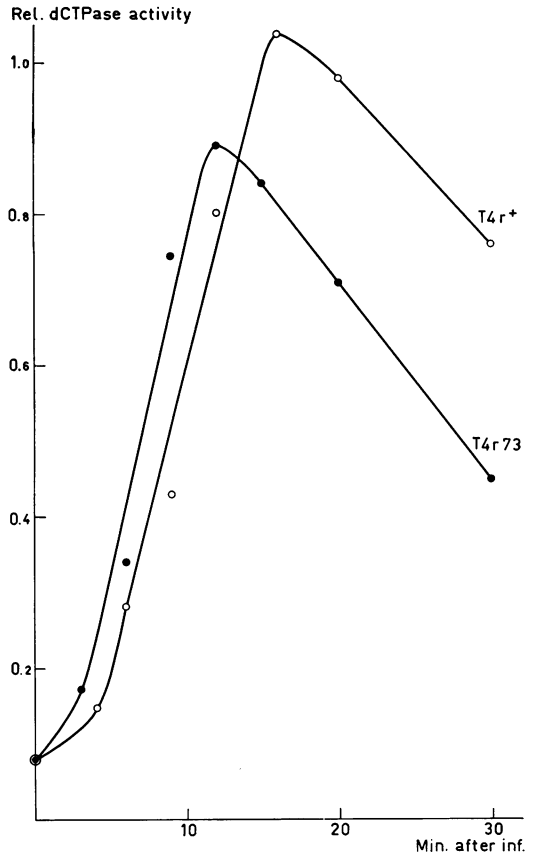

FIG. 1 *Deoxycytidine triphosphatase activity in Escherichia coli K(λ) infected with T4r$^+$ and T4r73 (rII).*

More detailed experiments were performed with rII-infected K(λ), always comparing them with r$^+$-infected K(λ).

The ratio between deoxyribonuclease activities of K(λ) infected with T4rII and r$^+$ is roughly the same throughout the latent period (Fig. 2).

Samples of K(λ) infected with T4r$^+$ and T4rII were mixed, and deoxyribonuclease activity was determined. The activity found was equal to the sum of the activity of each extract alone.

No increase in deoxyribonuclease activity could be seen when K(λ) was infected with rII phage in tryptone broth in the presence of 50 mM Mg^{++}, which partially reverses the rII block (11).

K(λ) was infected with r47 (cistron A) and r73 (cistron B), each at a multiplicity of 5. The deoxyribonuclease level was not higher than in cells infected with one of these rII mutants alone.

To test the possibility that the block against a continued DNA synthesis in rII-infected K(λ) depends on the early synthesis of some protein,

TABLE 2. *Deoxyribonuclease activity in different Escherichia coli strains infected with T4r⁺ or T4r*

Strain	Expt no.	Infection	Relative deoxyribonuclease activity	Deoxyribonuclease in r-infected cells/deoxyribonuclease in r⁺-infected cells
K(λ)	I	—	1	
		r⁺	4.9	
		r48(rI)	2.1	0.43
	II	—	1	
		r⁺	3.9	
		r47(rIIa)	2.0	0.51
	III	—	1	
		r⁺	4.6	
		r73(rIIb)	1.9	0.41
	IV	—	1	
		r⁺	4.9	
		rIII	2.4	0.49
K	I	—	1	
		r⁺	3.9	
		r48(rI)	1.7	0.44
	II	—	1	
		r⁺	3.9	
		r47(rIIa)	2.0	0.51
	III	—	1	
		r⁺	4.9	
		r73(rIIb)	1.5	0.31
	IV	—	1	
		r⁺	3.0	
		rIII	1.6	0.53
B	I	—	1	
		r⁺	2.5	
		r48(rI)	1.2	0.48
	II	—	1	
		r⁺	3.2	
		r47(rIIa)	2.1	0.66
	III	—	1	
		r⁺	2.5	
		r73(rIIb)	1.6	0.64
	IV	—	1	
		r⁺	2.5	
		rIII	1.4	0.56
Bc	I	—	1	
		r⁺	2.7	
		r48(rI)	1.9	0.71
	II	—	1	
		r⁺	2.7	
		r47(rIIa)	1.9	0.71
	III	—	1	
		r⁺	2.9	
		r73(rIIb)	1.8	0.62
	IV	—	1	
		r⁺	2.9	
		rIII	3.1	1.07

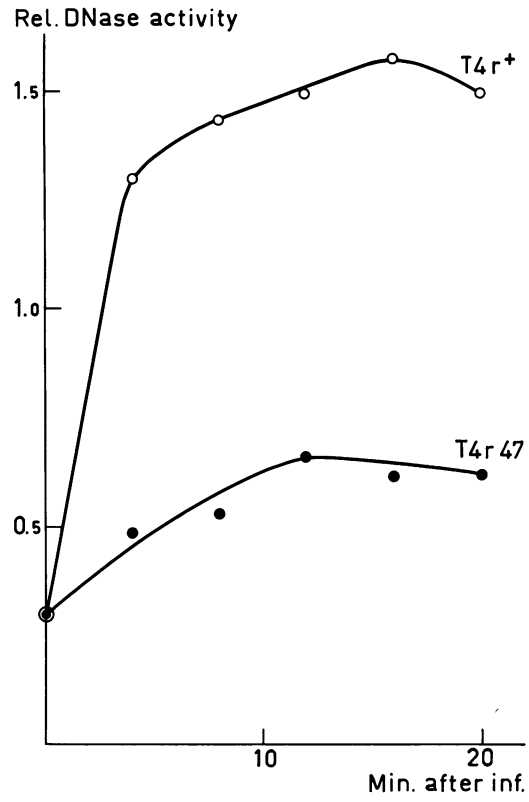

FIG. 2. *Deoxyribonuclease activity in Escherichia coli K(λ) infected with T4r⁺ and T4r47 (rII).*

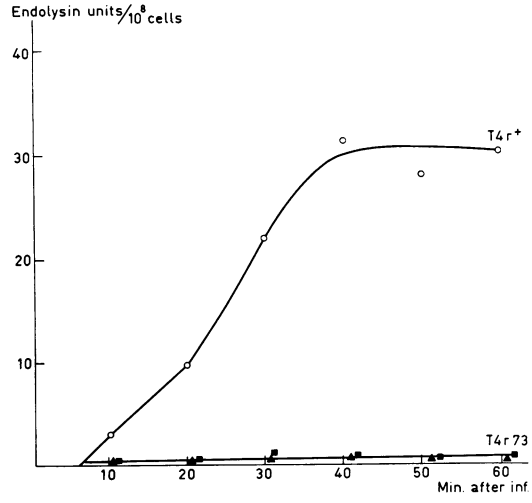

FIG. 3. *Endolysin activity in Escherichia coli K(λ) infected with T4r⁺ and T4r73 (rII). Two different experiments with T4r73 are shown.*

TABLE 3. *Number of phage equivalents of antiphage serum-blocking antigen per 2 × 10⁹ infected Escherichia coli K(λ)*

Min after infection	Antiserum-blocking phage equivalents	
	T4r+	T4r73
0	$<4 \times 10^8$	$<4 \times 10^8$
8	1.4×10^9	$<4 \times 10^8$
12	$\geqq 1.1 \times 10^{10}$	$<4 \times 10^8$
16	$\geqq 1.1 \times 10^{10}$	$<4 \times 10^8$
20	—	$<4 \times 10^8$
30	—	$<4 \times 10^8$

chloramphenicol (50 μg/ml) was added to a culture of K(λ) 3 min before infection to 12 min after infection, at intervals of 3 min. Incorporation of labeled thymidine into acid-insoluble products was measured. Without chloramphenicol or with the drug added later than 6 min after infection, incorporation was found both in r+- and rII-infected bacteria. In rII-infected cells, incorporation stopped at 12 min after infection, whereas it continued in r+-infected cells.

K(λ) infected with rII phage never showed lysozyme activity. The lysozyme of r+-infected K(λ) reached a maximal activity as late as 40 min after infection (Fig. 3).

Less than one phage equivalent of serum-blocking antigen per cell was found in samples of K(λ) taken between 4 and 30 min after infection with T4rII (Table 3).

DISCUSSION

Upon infection of sensitive bacteria with T-even bacteriophage, a number of phage-specific products are formed. These can be divided into two main groups: early functions, among which are found the enzymes necessary for the production of phage DNA, and late functions, such as phage structural components and lysozyme. The early functions appear immediately after infection, the late functions some 4 to 8 min later.

The experiments presented in this paper suggest that only early functions are expressed in rII-infected K(λ). Deoxycytidine triphosphatase, an early enzyme involved in phage DNA synthesis, is produced at the same rate up to about 12 min after infection of K(λ) with T4r+ or T4rII phage. Phage DNA is synthesized up to 10 to 15 min in rII-infected K(λ) (11, 13, 20). In contrast, no mature phage particles are produced, nor is any anti-phage serum-blocking antigen or phage lysozyme.

When sensitive bacteria are infected with phage of the T series, leakage of trichloroacetic acid-soluble, as well as trichloroacetic acid-insoluble, material occurs. Normally, this leakage stops at 5 to 7 min through the action of some resealing mechanism (15). This mechanism seems to be controlled by the phage, and in time this control would belong to the late functions. In rII-infected K(λ), no such mechanism seems to operate (9, 20).

We suggest that the block against rII phage in K(λ) is a composite of at least two factors: inability of the late functions to be expressed, and inability of any metabolic activity to proceed beyond 10 to 12 min after infection (11). As no late functions are expressed, the resealing mechanism is not working. At about 10 min, the continuous leakage has caused such an imbalance in the rII-infected K(λ) cell that all metabolic activity stops.

According to this model, the rII gene is of significance for the expression of phage-induced late functions in the host K(λ). No internal protein is found to be synthesized in rII-infected K(λ) when measured with immunological techniques (Mahler, Ph.D. Thesis, Brandeis Univ., Waltham, Mass., 1961). A shortage of polycations, which may be more or less compensated for in other hosts, could be the main constituent of the block against phage-induced late functions in rII-infected K(λ). It is known that the relative amounts of different polycations in the phage vary with the composition of the pool of cations in the host bacterium (2). Addition of polycations to rII-infected K(λ) permits growth of the phage (9).

An additional finding is the fact that extracts from *E. coli* infected with r mutants of T4 show less deoxyribonuclease activity than do extracts from T4r+-infected cells. Further purification of the extracts will probably give information regarding the nature of this difference.

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